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Indian Standard

METHOD FOR DETERMINATION OF PHOSPHATASE ACTIVITY IN MILK AND MILK PRODUCTS

PART I ROUTINE METHOD

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METHOD FOR DETERMINATION OF PHOSPHATASE ACTIVITY IN MILK AND MILK PRODUCTS

PART I ROUTINE METHOD

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Indian Standard

METHOD FOR DETERMINATION OF PHOSPHATASE ACTIVITY IN MILK AND MILK PRODUCTS

PART I ROUTINE METHOD

0. FOREWORD

0.1 This Indian Standard (Part I) was adopted by the Indian Standards Institution on 30 May 1977, after the draft finalized by the Dairy Products Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Phosphatase activity is determined to judge the efficiency of pasteurization of milk and milk products. To test whether the prescribed heat treatment was properly carried out, the treated milk or milk products is subjected to phosphatase test which helps to indicate the presence or absence of phosphatase enzyme. Phosphatase present in milk or milk products is destroyed by just about the same heat treatment necessary for the destruction of *Mycobacterium tuberculosis*, the most heat-resistant pathogen likely to be present in the products. The method is, therefore, applied for the control of proper pasteurization.

0.3 This standard is being issued in two parts. This part (Part I) covers the routine method for the determination of phosphatase activity which is carried out with special discs and a comparator and is used for day-to-day checks for the control of proper pasteurization of milk and milk products in a dairy. Part II covers the reference method which requires the use of a spectrophotometer and is usually applied in cases of dispute.

0.4 In the preparation of this standard, considerable assistance has been derived from various documents on the subject prepared by the International Organization for Standardization.

0.5 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

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*Rules for rounding off numerical values (*revised*).

1. SCOPE

1.1 This standard (Part I) specifies a routine method for the determination of the phosphatase activity in milk and milk powder; buttermilk and butter milkpowder; and whey and whey powder.

2. QUALITY OF REAGENTS

2.1 Unless specified otherwise, pure chemicals shall be employed in tests and distilled water (*see* IS : 1070-1977*) shall be used when the use of water as reagent is intended.

> Note — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

3. TERMINOLOGY

3.1 For the purpose of this standard, the phosphatase activity shall be the quantity of active alkaline phosphatase present in the product, expressed as the quantity of *p*-nitrophenol, in micrograms liberated under the specified conditions by 1 ml of the sample or in the case of dried products, by 1 ml of the reconstituted sample.

4. PRINCIPLE

4.1 The liquid sample or the reconstituted sample is diluted with a buffer at *p*H 10·2 and incubated at a temperature of 37°C for 2 hours. Any alkaline phosphatase present in the sample will, under these circumstances, liberate *p*-nitrophenol from added disodium *p*-nitrophenyl phosphate. The *p*-nitrophenol liberated is measured by direct comparison with standard colour discs in a simple comparator.

5. APPARATUS

5.1 Water-Bath — maintained at 37 ± 1°C, thermostatically controlled.

5.2 Comparator — with special discs of standard colour glasses calibrated in µg *p*-nitrophenol per ml milk, and 2 × 25 mm cells.

5.3 Test Tubes — of size 16 × 150 mm and rubber stoppers to fit.

5.4 Pipettes — 1, 5, and 10 ml.

5.5 Filter Paper — Whatman No. 2 or equivalent.

5.6 Litmus Paper

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*Specification for water for laboratory use (*second revision*).

6. REAGENTS

6.1 Sodium Carbonate-Bicarbonate Buffer — Dissolve 3·5 g of anhydrous sodium carbonate and 1·5 g of sodium bicarbonate in one litre of distilled water.

6.2 Buffer Substrate — Dissolve 1·5 g of disodium *p*-nitrophenyl phosphate in one litre of sodium carbonate-bicarbonate buffer (**6.1**). This solution is stable if stored in a refrigerator at 4°C or less for one month but a colour control test should be carried out on such stored solutions (*see* **7.4**).

6.3 Precipitants

6.3.1 *Zinc Sulphate* — Dissolve 30 g of zinc sulphate in 100 ml distilled water.

6.3.2 *Potassium Ferrocyanide* — Dissolve 15 g of potassium ferrocyanide in 100 ml distilled water.

7. GENERAL PRECAUTIONS

7.1 After use, test tubes should be emptied, rinsed in water, washed in hot water containing an alkaline detergent, followed by thorough rinsing in clean hot water. Finally they should be rinsed in distilled water and dried before use.

7.2 Pipettes should be thoroughly rinsed in clean cold water immediately after use, followed by rinsing in distilled water and dried before use.

7.3 The test tube stoppers should be thoroughly rinsed in hot water immediately after use, followed by boiling for 2 minutes in distilled water.

7.4 The buffer substrate solution (**6.2**) should remain stable for at least one month if stored in a refrigerator at 4°C or less. Any instability is denoted by the formation of a yellow colour. Whilst the test is always read against a boiled product control containing the same buffer substrate solution, it is recommended that the solution should not be used if it gives a colour reading in excess of 10 μg when read in a 25-mm cell in the comparator using distilled water in the 5-mm cell.

8. SAMPLING

8.1 While drawing and handling of the sample, same precautions as for bacteriological examination shall be observed.

9. PROCEDURE

9.1 Preparation of Sample

9.1.1 *Milk, Buttermilk and Whey*

9.1.1.1 Analysis should take place preferably directly after sampling. Otherwise keep the sample in a refrigerator but not for more than 48 hours.

9.1.1.2 Mix the sample carefully, if necessary with moderate heating. The temperature of mixing should not exceed 35°C.

9.1.2 *Milk Powder, Buttermilk Powder and Whey Powder* — Dissolve 10 g of the powder in 90 ml water. The temperature applied in dissolving the powder should not exceed 35°C.

9.1.3 *Neutralization of Sour Products* — Add to a sample of sour product, dilute sodium hydroxide solution, for example, 0·5 N or 0·1 N, until a drop of the product so treated, reacts neutral on litmus paper.

9.2 Determination

9.2.1 *Milk*

9.2.1.1 Pipette 5 ml of buffer substrate into a clean, dry test tube, followed by 1 ml of the milk to be tested. Stopper the tube, mix by inversion and place in the water-bath (*see* **5.1**).

9.2.1.2 At the same time place in the water-bath a control tube containing 5 ml of the buffer substrate and 1 ml of boiled milk of the same kind as that under test that is pasteurized homogenized, low fat.

9.2.1.3 After 2 hours, remove the tubes from the bath, invert each and read the colour developed using the comparator and special disc, the tube containing the boiled milk control being placed on the left of the stand and the tube containing the sample under test on the right. Record readings which lie between two standard colour discs by adding a plus (+) or minus (—) sign to the figure of the nearest standard.

NOTE — If artificial light is needed when taking these readings, an approved ' day light ' source of illumination must be used.

9.2.2 *Buttermilk, Whey, Milk Powder, Buttermilk Powder and Whey Powder*

9.2.2.1 Pipette 15 ml of buffer substrate into a clean, dry test tube, followed by 2 ml of the liquid product to be tested. Stopper the tube, mix by inversion and place in the water-bath (*see* **5.1**).

9.2.2.2 At the same time, place in the water-bath, a control tube containing 15 ml of buffer substrate and 2 ml of boiled liquid product of the same kind as under test.

9.2.2.3 After 2 hours remove both tubes from the water-bath, add 0·5 ml of zinc sulphate precipitant (**6.3.1**), replace the stopper, shake vigorously and allow to stand for 3 minutes. Add 0·5 ml of potassium ferrocyanide precipitant (**6.3.2**), mix thoroughly and filter through fluted Whatman No. 2 or equivalent filter paper and collect the clear filtrate in a clean test tube.

9.2.2.4 Transfer the filtrate to a 25 mm cell and compare against the filtrate of the boiled sample control in the comparator using the special disc (**5.2**).

10. REPORTING OF RESULTS

10.1 All samples giving readings in excess of 10 μg shall be recorded as unsatisfactory.

11. REPEATABILITY OF RESULTS

11.1 Results of duplicate determinations (results obtained simultaneously or in rapid succession by the same analyst) should not exceed 2 μg of *p*-nitrophenol over the critical range 0 to 14 μg *p*-nitrophenol.

(*Continued from page* 2)

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